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# Routine clinical inspections in Norwegian marine salmonid sites: A key role in surveillance for freedom from pathogenic viral haemorrhagic septicaemia (VHS)

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ABSTRACT

Since the mid-1980s, clinical inspections of aquaculture sites carried out on a regular basis by authorized veterinarians and fish health biologists (known as fish health services: FHS) have been an essential part of aquatic animal health surveillance in Norway. The aims of the present study were (1) to evaluate the performance of FHS routine clinical inspections for the detection of VHS and (2) to explore the effectiveness of risk-based prioritisation of FHS inspections for demonstrating freedom from VHS in marine salmonid sites in Norway. A stochastic simulation model was developed to estimate site sensitivity (SeS), population sensitivity (SeP), and probability of freedom (PFree). The estimation of SeS takes into consideration the probability that FHS submit samples if a site is infected, the probability that a sample is tested if submitted, the effective probability of infection in fish with clinical signs, laboratory test sensitivity, and the number of tested samples. SeP and PFree were estimated on a monthly basis over a 12 month period for six alternative surveillance scenarios and included the risk factors: region, species, area production density, and biosecurity level. Model results indicate that the current surveillance system, based on routine inspections by the FHS has a high capability for detecting VHS and that there is a high probability of freedom from VHS in Norwegian marine farmed salmonids (PFree >95%). Sensitivity analysis identified the probabilities that samples are submitted and submitted samples are tested, as the most influential input variables. The model provides a supporting tool for evaluation of potential changes in the surveillance strategy, and can be viewed as a platform for similar exotic viral infectious diseases in marine salmonid farming in Norway, if they share similar risk factors.



## 1. Introduction

Surveillance for freedom from disease is an important prerequisite for disease control programmes and safe international trade of animals. A number of approaches and methods used to provide evidence of freedom from diseases have been continuously developed over the last decades. One such method is risk-based surveillance, which is a well-recognized method that aims to improve the cost-effectiveness of surveillance systems for disease detection or prove freedom from disease, and to provide support for both strategic and operational decision making (Stärk et al., 2006). According to the current EU Council Directive 2006/88/EC on aquatic animal health, a country that is declared free from listed diseases may maintain its disease-free status without carrying out targeted surveillance. However, this requires that conditions conducive to clinical expression of the disease in question exist, and that a risk-based surveillance scheme is in place on aquaculture sites in order to detect listed diseases and investigate increased mortality events. Even though risk-based surveillance has been widely used for diseases in terrestrial animals (Christensen et al., 2011; Wahlstrom et al., 2011; Goutard et al., 2012; Velasova et al., 2012; Welby et al., 2012; Boklund et al., 2013; Calvo-Artavia et al., 2013; Frössling et al., 2013; Oidtmann et al., 2013), only a very few examples have been applied to surveillance systems in farmed aquatic animals. Peer-reviewed literature presenting and evaluating examples or models of risk-based surveillance schemes in farmed aquatic animals is, to our knowledge, limited (Oidtmann et al., 2013; Gustafson et al., 2014; Marques et al., 2015).

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The annual salmonid production in Norway reached 1.3 million metric tons round weight in 2014 (source: Directorate of Fisheries, www.fiskeridir.no). During a production cycle, juvenile salmonids are kept in freshwater sites until smoltification, and are thereafter moved to marine grow-out sites, where the fish are kept in sea cages. These are high-density populations where each cage can keep up to 100 000 individuals or more, presenting challenges to fish inspection and representative sampling for health surveillance.

In Norway, health surveillance and monitoring programmes for infectious and non-infectious diseases of fish-farming sites have been implemented since the mid -1980s. The system is based on regular health inspections carried out by authorized fish health services (FHS), who are trained veterinarians or fish health biologists. The inspections carried out by FHS are conducted according to the requirements and criteria described in Norwegian regulations (Ministry of Trade Industry and Fisheries, 2008). Briefly, FHS inspections should be based on an assessment of the risks of infection, disease development in the production system, and spread to other sites. In a normal situation, all farmed salmonid grow-out sites are inspected a minimum of six times per year. Additional clinical inspections may be required at the time of sea transfer of smolts and in case of increased mortality or suspicion of disease. The routine inspections should be spread approximately equally throughout the year.

Viral haemorrhagic septicaemia (VHS) is recognized as a viral disease of farmed salmonids and non-salmonids, and in a range of wild fish, in both freshwater and marine environments. VHS is a serious viral disease, responsible for significant losses in rainbow trout (*Oncorhynchus mykiss,* RBT), in particular (Skall et al., 2005; Smail and Snow, 2011; Office International des Epizooties, 2012). The disease is caused by VHS virus (VHSV), a virus in the genus *Novirhabdovirus* of the family Rhabdoviridae (Walker et al., 2000). Phylogenetic studies of VHSV have identified four geographically distributed genotypes (I–IV), including freshwater and marine VHSV variants (Snow et al., 1999, 2004; Einer-Jensen et al., 2004, 2005). For this study, we focus on VHSV genotypes that cause clinical disease in marine farmed salmonids, as described by the OIE (Office International des Epizooties, 2012).

Outbreaks of VHS are characterized by nonspecific clinical signs in the early stage of infection followed by a rapid onset of mortality. Freshwater VHSV variants, in particular, cause severe disease in RBT (Office International des Epizooties, 2012; Olesen and Skall, 2013). VHSV originating from wild marine fish has generally not been associated with disease in RBT (Skall et al., 2005). Reports on susceptibility of Atlantic salmon (*Salmo salar*, AS) to marine VHSV are limited, and major disease outbreaks in AS caused by freshwater or marine VHSV isolates have not been reported to date. However, AS are classified as susceptible for VHS by OIE (King et al., 2001; European Food Safety Authority, 2008; Office International des Epizooties, 2012).

VHS in RBT was reported in Norway between 1964 and 1974, but was successfully eradicated (Håstein et al., 1968; Lorenzen and Olesen, 1999). Norway was approved free from VHS according to EU legislation in 1994 (EFTA Surveillance Authority, 1994), and, since then, has operated a surveillance programme following the EU guidelines, documenting the absence of VHSV at aquaculture sites in order to maintain the VHS-free status. However, in 2007, VHS was diagnosed in RBT at a marine site in south-western Norway. The VHSV associated with this outbreak was classified as genotype III, a marine genotype that was demonstrated to be pathogenic to RBT for the first time (Dale et al., 2009). When the diagnosis of VHS was confirmed in 2007, Norway's VHS-free status was temporarily suspended. Measures to eliminate the disease and to prevent its spread were immediately implemented by the Norwegian Food Safety Authority (NFSA) and, with the exception of the VHS outbreak area, Norway was again recognized as an approved VHS-free zone in May 2008 (EFTA Surveillance Authority, 2008). Norway regained its VHS-free status for the whole country in 2011.

Until 2009, the surveillance programme for VHS in Norway was carried out by the NFSA in accordance with the Council Directive (CD) 91/67/EEC. This programme was based on sampling 30 fish from all AS and RBT sites during a two-year period, and analysing for VHSV by cell-culture. According to the CD, inspection and sampling should be carried out when the water temperature was below 14 °C. Fish showing clinical signs of disease should be sampled if present. In 2008, a total of 1 398 pooled samples (from 13 980 individual fish) collected from 444 sites in Norway were examined by cell culture (Hellberg et al., 2009). A continuation of this program, where individual samples are tested by PCR, is estimated to 652 thousand euros at today's cost (this equates to 47 euro per individual sample, cost of inspections are not included). Due to the increasing demand for more cost-effective surveillance strategies, the Norwegian surveillance programme for VHS was modified in 2009 towards a risk-based approach where all RBT sites and a proportion of AS sites were sampled (Lyngstad et al., 2010). In this new regime, only fish showing clinical signs of disease were sampled by the FHS. In 2014, 1490 individual samples were investigated by real time RT-PCR (Gjevre et al., 2015).

Routine clinical inspection carried out by FHS is considered a key factor for early detection of VHS in marine farmed salmonids. The aims of this study were (1) to evaluate the performance of FHS routine clinical inspections for the detection of VHS and (2) to explore the effectiveness of risk-based prioritisation of FHS inspections for demonstrating freedom from VHS in marine farmed salmonids in Norway.

## 2. Materials and methods

### 2.1. Model overview

The core surveillance activity to be modelled here is the routine clinical inspections by FHS on farmed salmonids sites. During each visit, the FHS officer inspects all production units (sea cages) within a site and carries out post mortem investigation on selected dead or moribund fish. The FHS officer decides whether or not to submit samples to an authorized laboratory, either the Norwegian Veterinary Institute (NVI) or a private one for further investigation by PCR or histopathology based on clinical or macro-pathological findings. In case of suspicion of VHS, samples have to be submitted to the National Reference Laboratory, *i.e.* NVI for confirmation.

The performance of the routine clinical inspections was evaluated using a stochastic simulation model in accordance with the methods described by Martin et al. (2007). Model outputs were: the confidence of VHS detection, if it was present at given design prevalences *i.e.* site sensitivity (SeS) and population sensitivity (SeP); and the probability that the population is free from VHS at the specified design prevalence given that there was no VHS detected by the surveillance system, *i.e.* probability of freedom (PFree). SeP and PFree were estimated on a monthly basis over a 12 month period for six alternative surveillance scenarios, incorporating four risk factors: region, species, area production density (APD), and biosecurity level (BSL), as described in subsequent sections. The case definition for this analysis was a VHS diagnosis confirmed by the NFSA, according to the diagnostic criteria described by OIE (Office International des Epizooties, 2012). The time period used in the model was one month to allow flexibility in the frequency of modelled FHS inspections and because of the acute nature of VHS.

Data management and analyses were performed using R version 3.2.2 (R Development Core Team, 2011). Monte Carlo simulation with 10 000 iterations was run for the simulation of each scenario. Stochasticity was incorporated by input parameters being

**Table 1**
Description of model input parameters and distributions used to estimate site sensitivity (SeS), population sensitivity (SeP) and probability of freedom (PFree).

| Input parameter | Value | Explanation and source of data |
|---|---|---|
| $p^*$ | 0.15 | Design prevalence within a site. 15 % of total number of fish was used. |
| $P^*$ | 4 sites/# active sites | Design prevalence at site level, corresponding to 4 sites in the total population of active sites per month, 0.7% for month 1. |
| Prior Pfree | 0.5 | Prior probability of freedom. |
| PIntro | 0.004 | Probability of introduction. Value is based on data available in the 240 months since Norway was approved as free from viral haemorrhagic septicaemia (VHS, EFTA Surveillance Authority, 1994; Dale et al., 2009). |
| Sampled | Pert (0.6, 0.7, 0.8) | Probability that rainbow trout (RBT) or Atlantic salmon (AS) are sampled and submitted, given that the population is infected at design prevalence. Authors' best guess. |
| Tested | Pert (0.8, 0.9, 0.95) | Probability that samples are tested given that the population is infected and sampled submitted. Authors' best guess. |
| SePCR | Beta (54,7) | Sensitivity of real-time PCR test, Jonstrup et al. (2013). |
| TpRBT | Beta (26,7) | Probability of clinical signs in VHS-infected RBT. Derived from experiments in Dale et al. (2009). |
| FpRBT | Empirical data | Probability of clinical signs in VHS-uninfected RBT were obtained from the monthly reports on production statistics to the Norwegian authorities during 2014, as described in Kristoffersen et al. (2009). |
| TpAS | Beta (18,59) | Probability of clinical signs in VHS-infected AS. Derived from experiments in Dale et al. (2009). |
| FpAS | Empirical data | Probability of clinical signs in VHS-uninfected AS were obtained from the monthly reports on production statistics to the Norwegian authorities during 2014, as described in Kristoffersen et al. (2009). |
| $n$ | 5:20 | Number of fish samples submitted to laboratory. Based on current practice, we use a simulated distribution with sampling range (5:20) and a decreasing sampling frequency (50,20,10,3,3,2,2,2,1,1,1,1,1,1,1,1). |

randomly drawn from specified probability distributions. Input distributions are summarised in Tables 1 and 2 described in more detail in the following sections.

### 2.2. Population at risk

The population at risk for this analysis consisted of all active marine grow-out sites, *i.e.* sites with fish for human consumption or brood stock, with RBT and/or AS in 2014. An active site was defined as a site stocked with fish for at least 1 month in 2014, based on production statistics reported to the Norwegian Authorities, as described in Kristoffersen et al. (2009). The numbers of active sites varied from month to month as individual sites were stocked or harvested. Sites stocked with both AS and RBT (mixed sites) were categorized as RBT sites.

### 2.3. Site sensitivity (SeS)

SeS differed between species *i* (RBT and AS), and was calculated as

$$\mathrm{SeS_i} = \mathrm{Sampled} \times \mathrm{Tested} \times \left(1 - (1 - \mathrm{SePCR} \times \mathrm{EPIF_i})^n\right)$$

where "Sampled" is the probability that samples from moribund or fresh dead fish are submitted for testing, given that the site is infected. Model input for "Sampled" was based on the competence of FHS personnel in recognizing fish with VHS clinical signs, and on previous experience showing that direct sampling of moribund or suspicious fish is much more efficient for VHS detection than random sampling of apparently healthy fish. Since quantitative information on the probability that fish samples are submitted (given that the site is infected) is unknown, we used a Pert distribution with a conservative range (minimum = 0.60, median = 0.70, and maximum = 0.80), for both RBT and AS."Tested" is the probability that samples submitted from an infected site where fish exhibit clinical signs will be tested at the laboratory for VHSV. Operating procedures at the NVI require that when VHS is suspected or is a relevant differential diagnosis, samples are to be tested for VHSV. The probability of testing is, therefore, assumed to be high and "Tested" was modelled as a Pert distribution with minimum = 0.80, median = 0.90, and maximum = 0.95, for both RBT and AS."SePCR" is the sensitivity of the laboratory test (a real time RT PCR test) for VHSV that is currently being used in the ongoing VHS surveillance programme in Norway (Gjevre et al., 2015). The PCR test has an estimated sensitivity of 0.9 (53 positive of 59 infected fish tested) and specificity close to one (Jonstrup et al., 2013). This parameter ("SePCR") was therefore modelled using a Beta probability distribution (54, 7), with a median of 0.89 and the 5th percentile of 0.81, based on the published estimate in Jonstrup et al., (2013)."EPIF" is the effective probability of infection among fish with clinical signs. Because clinical signs of VHS are non-specific and may be expressed both in infected and uninfected fish, we estimated EPIF as the positive predictive value of the occurrence of clinical signs for VHSV infection for each species *i* (RBT and AS), using Bayes theorem:

$$\mathrm{EPIF_i} = \frac{p^* \times T\mathrm{p_i}}{p^* \times T\mathrm{p_i} + (1 - p^*) \times F\mathrm{p_i}}$$

where "$p^*$" is the fish-level design prevalence on an infected site. The prevalence of VHSV-infected fish within an infected site depends on genotype and strain. A high prevalence (in a short period of time) is expected in a naïve population (Office International des Epizooties, 2012). Because VHS is considered to be a highly contagious infection, a design prevalence of 15 % was used."*T*p" is the probability of clinical signs (*i.e.* dead or moribund fish, weak fish, fish with signs of systemic infection, or fish with abnormal behaviour) in VHSV-infected fish, and "*F*p" is the probability of clinical signs in VHSV-uninfected RBT and AS. "*T*p" was derived from experimental trials (Dale et al., 2009) where 81% (25/31) mortality was found in VHSV-infected RBT, and 23% (17/75) mortality was found in VHS-infected AS after 10 days of trial. To allow for uncertainty for these estimates, they were modelled as Beta (26,7) and Beta (18, 59) distributions for "*T*p" of RBT and AS, respectively, based on the above data. "*F*p" was estimated by sampling from distributions of reported values on monthly site mortality in 2014 (production data). Median and mean monthly mortality in RBT sites were 0.7% and 1.5% (the 95% range was given by 0.1% and 6.1%) and 0.4% and 1.1% (0.07%, 4.2%) in AS sites.

The number of samples tested is denoted by "*n*" and, based on current practice, the input distribution ranged from 5 to 20 samples with a decreasing sampling frequency (Table 1).

### 2.4. Site level risk factors for VHS infection

At the population level, region, species, area production density (APD), and biosecurity level (BSL) were identified as risk factors for VHS (Table 2).

For region, sites located in northern Norway (Region North) were considered to have a lower risk of acquiring VHS than sites in southern Norway (Region South, Fig. 1). This assumption is based on historical data on outbreaks of VHS that occurred in southern Norway in the 1960s and 1970s (Håstein et al., 1968) and

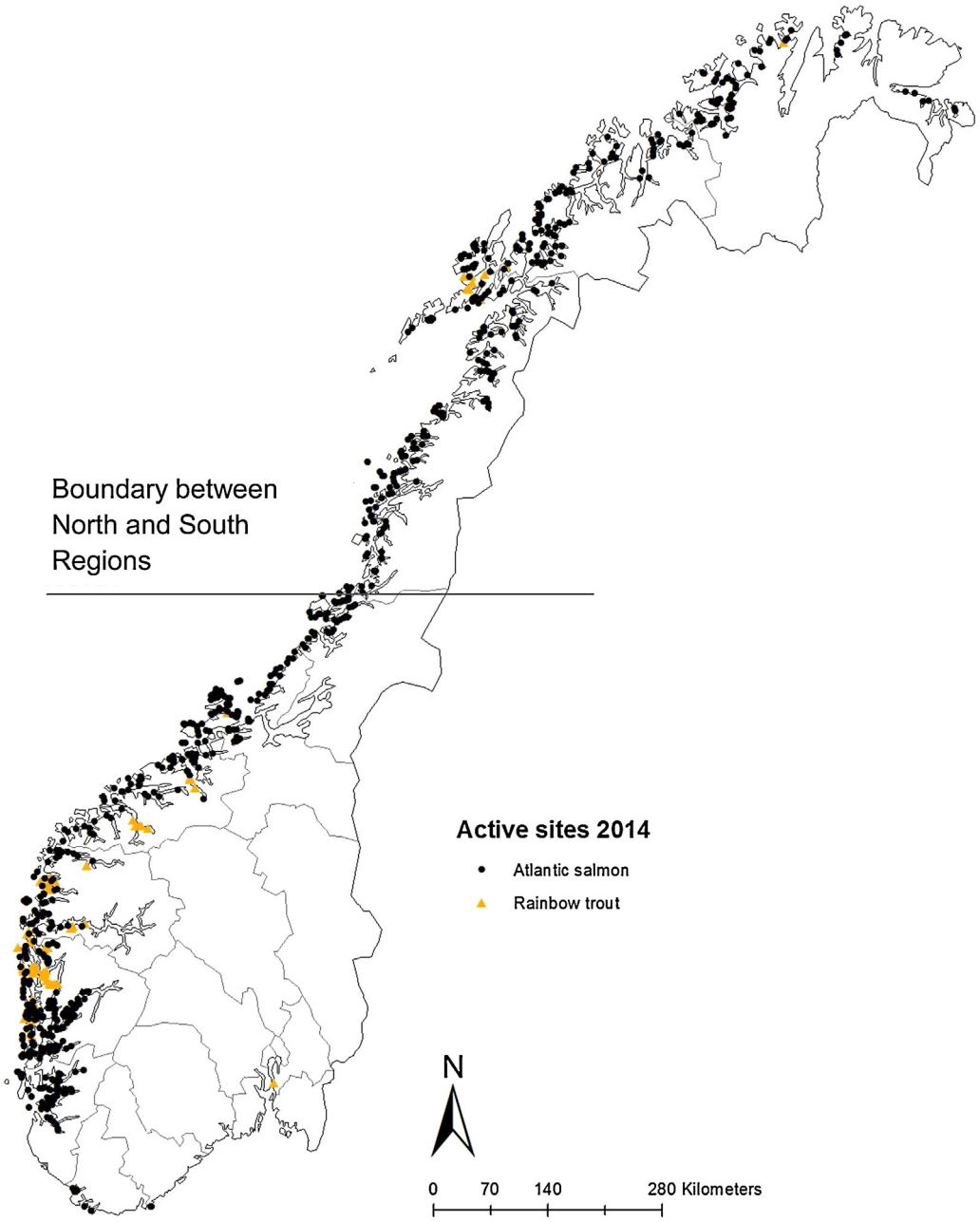


**Fig. 1.** Map of Norway showing active sites with rainbow trout (RBT, yellow triangle) and Atlantic salmon (AS, black circle). The boundary between Region South and Region North is marked with a black line. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

in south-western Norway in 2007 (Dale et al., 2009). In addition, VHSV in wild marine fish in the North Sea and the coastal regions of Norway is primarily found in the south (Brudeseth and Evensen, 2002; Skall et al., 2005; Sandlund et al., 2014). A most likely relative risk (RR) of two was assumed between the two regions and was modelled as a Pert (1, 2, 5) distribution to allow for uncertainty.

The risk associated with species was based on results from infection trials in which the susceptibilities of RBT and AS to VHSV were examined. One immersion trial using the marine VHSV strain found in Norway in 2007, reported mortality in all RBT groups and no mortality in the AS groups. Mortality of AS was only observed after intraperitoneal injection (Dale et al., 2009). In another immersion trial, AS was shown to be susceptible to a highly pathogenic VHSV strain, with VHSV detected in one of six fish groups (16% of the fish groups, King et al., 2001). Based on these findings we assumed that the probability of VHS in an exposed population was 100% for RBT and 16% or less for AS, giving a RR of 6.25 for RBT *versus* AS. To allow for uncertainty a Pert (2, 6.5, 10) distribution was used.

The risk associated with APD was based on general assumptions of density-dependent transmission of infectious diseases (Krkošek, 2010), supported by results from studies on other infectious viral or parasitic diseases in salmonid farming (Kristoffersen et al., 2009; Mardones et al., 2009; Aldrin et al., 2010; Bang Jensen et al., 2012). The APD for Norwegian salmonid farming sites was calculated for each site as a kernel density of average monthly biomass on the site and surrounding sites within 40 km as described in Jansen et al. (2012). In the model, active sites were categorized into high-density and low-density subgroups by using the 3rd quartile of APD over the range of sites as a cut off. RR of two was assumed for the sites

**Table 2**
Proportion of population in different groups and the respective relative risk (RR) estimates with regard to risk factors for viral haemorrhagic septicaemia (VHS) included in the model used to estimate population sensitivity (SeP) and probability of freedom (PFree).

| Risk factor | Population proportions | | Relative risks (RR) | | Source of data |
|---|---|---|---|---|---|
| | Description | Value | Description | Value | |
| Region | Region South | 0.63 | RR Region | Pert (1, 2, 5) | Production statistics[a] |
| | Region North | 0.37 | Region South compared to Region North | | Hästein et al. (1968); Brudeseth and Evensen (2002); Skall et al. (2005); Dale et al. (2009); Sandlund et al. (2013) |
| Species | Rainbow trout (RBT) | 0.09 | RR Species RBT compared to | Pert (2, 6.5, 10) | Production statistics[a] |
| | Atlantic salmon (AS) | 0.91 | AS | | Experiments described in King et al. (2001) and Dale et al. (2009) |
| Area production density (APD) | High APD | 0.25 | RR APD high compared to low | Pert (1, 2, 5) | Production statistics[a] |
| | Low APD | 0.75 | | | Kristoffersen et al. (2009); Mardones et al. (2009); Aldrin et al. (2010); Krkošek (2010); Bang Jensen et al. (2012) Authors' best guess |
| Biosecurity level (BSL) | Low BSL | 0.1 | RR BSL low low compared to high | Pert (2.5, 3, 5) | Population proportions were based on hypothetical population proportion due to lack of available data. Authors' best guess |
| | Medium BSL | 0.4 | RR BSL med | Pert (1.5, 2, 2.5) | |
| | High BSL | 0.5 | medium compared to high | | |

[a] Source of population proportions. Production statistics is the monthly reports on production statistics to the Norwegian authorities during 2014 as described in Kristoffersen et al. (2009).

with high APD compared to sites with low APD, and a Pert (1, 2, 5) distribution was used to allow for uncertainty of RR.

The risk associated with BSL was based on hypothetical RR and population proportion due to lack of data. This node was included in order to demonstrate the potential impact of the level of biosecurity on sites, including different unknown components like daily management, presence of wild fish, and movement of fish between sites. A Pert (2.5, 3, 5) was used for RR of low BSL compared to high BSL, and a Pert (1.5, 2, 2.5) distribution was used for RR of medium BSL compared to high BSL (Table 2).

For each of four risk factors, R1–R4 (*i.e.* region, species, APD and BSL), adjusted relative risks (ARR) for month $t$ were calculated by adjusting RR for the differences in population proportion for all subgroups ($l$) of the specific risk factor as:

$$\mathrm{ARR}_{\mathrm{l},t} = \frac{\mathrm{RR}_{1,t}}{\sum_{l=1}^{\max l}\left(\mathrm{RR}_{\mathrm{l},t} \times \mathrm{PopPr}_{\mathrm{l},t}\right)}$$

where "RR" is the relative risk and "PopPr" is the population proportion in the respective risk subgroup l. Population proportions for each risk factor were estimated at the population level from production data.

The effective probability of infection at site level "EPIS" was calculated for all combinations of R1–R4 (resulting in 24 risk strata) and each month $t$ as:

$$\mathrm{EPIS}_{\mathrm{R1,R2,R3,R4},t} = \mathrm{P}_t^* \times \prod_{\mathrm{R1}=1}^{2} \times \prod_{\mathrm{R2}=1}^{2} \times \prod_{\mathrm{R3}=1}^{2} \times \prod_{\mathrm{R4}=1}^{3} \mathrm{ARR}_{\mathrm{R1,R2,R3,R4},t}$$

where "$P^*$" is the design prevalence at site level. In order to demonstrate freedom from VHS and support the purpose of early detection, we used a design prevalence of four sites in the total population of active sites per month. This corresponds to approximately 0.7% per month. The exact value varied, depending on the number of available active sites each month. Independence between the four risk factors was assumed.

### 2.5. Population sensitivity (SeP)

SeP was estimated as one minus the product of the probability of a negative result across all 24 risk strata using a binomial approximation to the hypergeometric distribution, adapted from (MacDiarmid, 1988) for each $t$ month:

$$\mathrm{SeP}_t = 1 - \prod_{j=1}^{24}\left(1 - \mathrm{SeS}_j \times \frac{n_{\mathrm{j,t}}}{N_{\mathrm{j,t}}}\right)^{\mathrm{EPIS}_{\mathrm{j,t}} \times N_{\mathrm{j,t}}}$$

where $j$ indicates a risk stratum ranging from 1 to 24, based on all possible combinations of R1, R2, R3, R4.″$\mathrm{SeS}_j$″ is the site sensitivity as described in Section 2.3 (species given by risk stratum $j$). The approximation to the hypergeometric distribution was used because of the small number of sites in the risk group.″$n_{j,t}$″ is the number of sites inspected in risk stratum $j$ and month $t$, according to the scenario. The value for n for each stratum and month was estimated as a binomial function of N and the frequency of inspection for the relevant risk stratum and scenario.″$N''_{j,t}$ is the number of active sites in risk stratum $j$ and month $t$ as calculated from population proportion data.“$\mathrm{EPIS}_{\mathrm{j,t}}$” is the effective probability of infection at site level in risk stratum $j$ and month $t$ as described in Section 2.4.

### 2.6. Estimating probability of freedom (PFree)

The probability of freedom (PFree) was estimated for month $t$ as:

$$\mathrm{PFree}_t = \frac{\mathrm{PriorPFree}_t}{1 - \mathrm{SeP}_t \times (1 - \mathrm{PriorPFree}_t)}$$

where "$SeP_t$" is population sensitivity for month $t$, assuming population specificity of 100 %. "Prior PFree" was set to be 50% for the first month, and calculated for successive time periods $t$ as:

$$PriorPFree_t = 1 - [1 - PFree_{t-1} + PIntro_t - ((1 - PFree_{t-1}) \times PIntro_t)]$$

where "PIntro" is the probability of introduction, and was assumed to be 0.4 %, based on data available from the 240 months since Norway was approved free from VHS (EFTA Surveillance Authority, 1994). An estimated PIntro of 0.4% corresponds to 1 introduction in 240 months.

### 2.7. Model scenarios

In order to evaluate differences in frequency of inspections in the different risk strata, the following six scenarios were simulated: Scenario I when six annual inspections are conducted in all marine RBT and AS sites; Scenario II when three annual inspections are conducted in all marine RBT and AS sites; Scenario III when six annual inspections are conducted in RBT sites and one annual visit is conducted in AS sites; Scenario IV when six annual inspections are conducted in sites in Region South and three are conducted in sites in Region North; Scenario V when six, four and zero inspections are conducted in sites with low, medium, and high BSL respectively; and Scenario VI with six annual inspections in sites with high APD and three in sites with low APD.

### 2.8. Sensitivity analysis of model input variables

In order to identify the most influential variables in the model, we used a simple linear regression model to estimate standardised regression coefficients for all input variables, with the SeP for month one as the dependent variable. The change in SeP (and 95% confidence interval) for a one standard deviation change in the input variable was estimated and compared for all input variables and for all six scenarios.

## 3. Results

In 2014, there were a total of 801 active marine salmonid sites, of which 71 sites were growing RBT alone or RBT mixed with other species, and 730 sites rearing AS alone. The median number of fish per site was ~452 000 (the 95% interval was given by 33 000 and 989 000) for RBT sites, and ~682 000 (84 000, 1 566 000) for sites with AS.

Results of the model showed that the median SeS estimates were 0.62 for both RBT and AS. The 95% probability interval (PI) was between 0.55 and 0.70 for RBT, and between 0.53 and 0.70 for AS. The median EPIF estimates were 0.95 for RBT (95% PI 0.61 and 0.99), and 0.92 for AS (0.35 and 0.99).

The estimated median SeP varied by different surveillance scenarios, but showed little variation from month to month within the same scenario. SeP estimates were 0.67 (95% PI 0.60 and 0.73), 0.39 (0.33, 0.46), 0.21 (0.16, 0.26), 0.64 (0.57, 0.70), 0.29 (0.23, 0.35), and 0.47 (0.40, 0.54) for month one for Scenarios I–VI, respectively (Fig. 2 ).

Scenario I, having the highest frequency of clinical inspection, provided the highest probability of detection (SeP estimates per month). Scenario III, having the lowest frequency of clinical inspection, provided the lowest SeP estimate.

A high probability of freedom (estimated median PFree >95%) was achieved within three months for Scenarios I and IV, within five months for Scenario VI, in seven months for Scenario II, and 10 months for Scenario V. The estimated median PFree of Scenario III was 0.93 after 12 months of inspections (Fig. 2).

The estimated change in SeP for month one with one unit standard deviation change in each input variable, is given in Fig. 3. Variables with confidence limits that included zero were non-significant. The analysis showed that the probabilities that RBT are sampled and that the samples are tested, given that the site is infected were the two most influential input variables in the model for all six scenarios. Other significant input variables were the probabilities that AS are sampled and tested, the RRs of region, species, BSL, the probability of clinical signs in infected AS, the probabilities of clinical signs in uninfected RBT and AS and the PCR test sensitivity. However, these had minor effects on the estimated population sensitivity. The estimated changes in SeP for changes in the probabilities of clinical signs in uninfected RBT and AS were slightly negative in all scenarios. This indicates that the higher the false positive rate, the lower would be the EPIF estimate and, consequently, site sensitivity.

## 4. Discussion

The main results from the present model indicate that the current surveillance system based on routine clinical inspections by the competent fish health service (FHS) has a high capability for detecting VHS. The results show that within three to 10 months the surveillance has achieved a high probability of freedom (PFree >95%) from VHS in Norwegian marine farmed salmonids for five of the six scenarios. Exploring risk-based surveillance highlights the importance of frequent visits in high-risk strata of the population. Scenario IV, which was based on six annual visits per year in Region South and three in Region North, was identified as the most cost-effective approach for documenting freedom from VHS (PFree >95% achieved after only three months). Because the system of routine clinical inspections carried out by FHS has been in place in Norway since the mid- 1980s the accumulated confidence in freedom from VHS is very high.

VHS is a highly transmissible infection that is expected to display clinical signs and rapid mortality in the early stages of infection in a naive population (Office International des Epizooties, 2012). VHS is, therefore, a disease suitable for a surveillance scheme based on clinical inspections followed by laboratory testing of suspicious samples. Experience has also shown that sampling of moribund fish and/or fish with clinical signs by competent professionals are much more efficient at VHS detection than random sampling of apparently healthy fish (Lyngstad et al., 2008; N. J. Olesen, DTU-VET, pers.comm).

The estimation of marine site sensitivity (SeS) included the probability that FHS submit samples if a site is infected, the probability that a sample is tested if submitted, the EPIF in RBT and AS with clinical signs, laboratory test sensitivity, and the number of tested samples. The SeS estimates were similar for both RBT and AS, reflecting the importance of sampling and testing fish with clinical signs.

The population was divided into risk strata based on differences in risk of VHS in order to explore the effectiveness of risk based surveillance. Region was included due to experience and previous history that indicated different regional exposures to VHSV. Species were differentiated based on the susceptibility to infection, as current data indicate that RBT are more susceptible to VHSV than AS (Smail and Snow, 2011). Area production density (APD) was included because a number of studies have demonstrated that high-production density is an important factor in spread of infectious pathogens in the aquatic environment (Kristoffersen et al., 2009; Mardones et al., 2009; Aldrin et al., 2010; Bang Jensen et al., 2012). APD in the present model illustrated the possibility for improvement of the surveillance programme, as the results showed that prioritizing sites from high-density areas can reduce costs

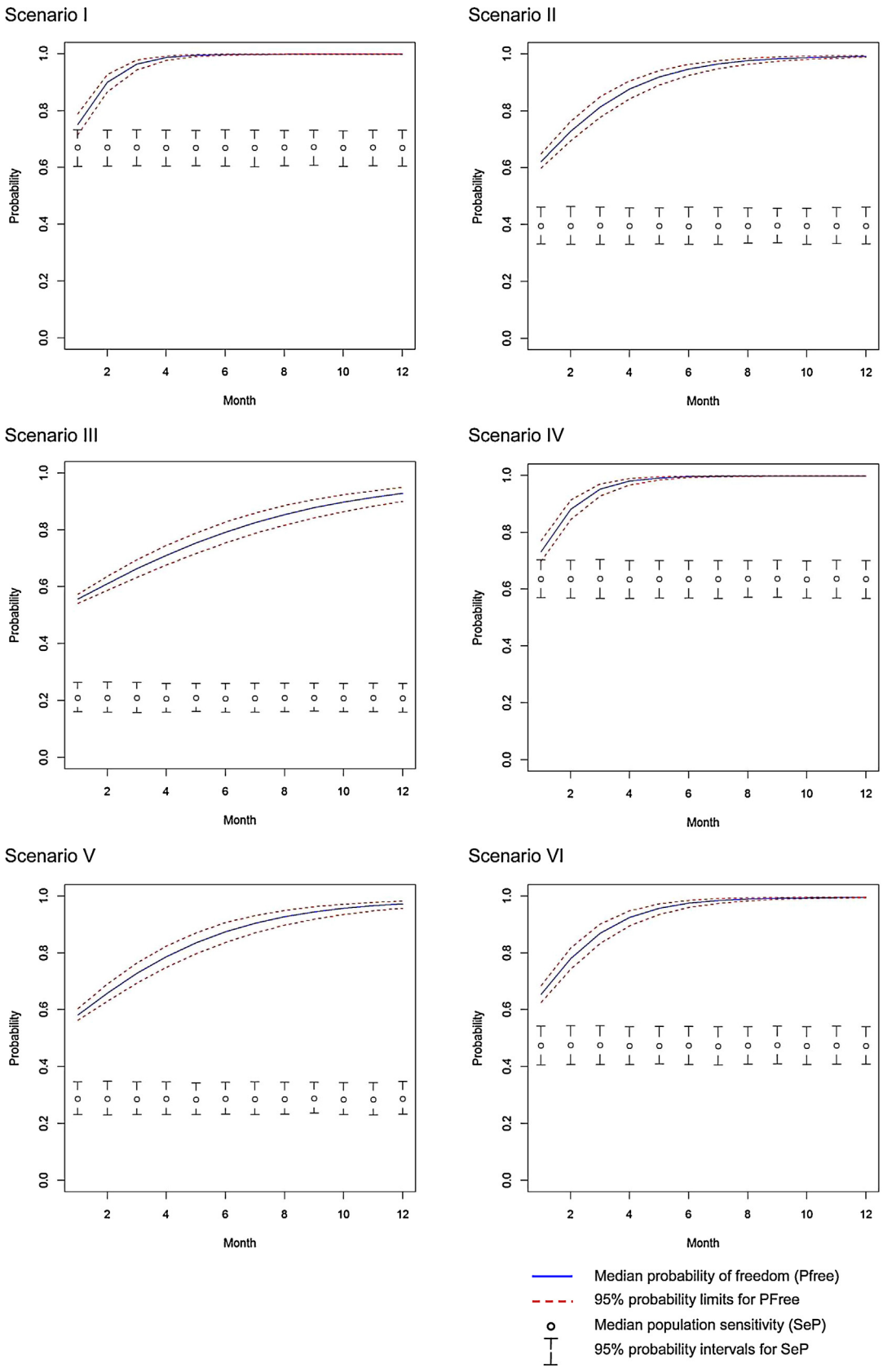


**Fig. 2.** Estimated population sensitivity (SeP) and probability of freedom (PFree) over a 12 month period for viral haemorrhagic septicaemia (VHS) for each of the six alternative surveillance scenarios: Scenario I with six annual inspections in all marine RBT and AS sites, Scenario II with three annual inspections in all marine RBT and AS sites, Scenario III with six annual inspections in RBT sites and one annual visit in AS sites, Scenario IV with six annual inspections in sites in Region South and three in sites in Region North, Scenario V with six, four and zero inspections in sites with low, medium and high BSL, respectively, and Scenario VI with six annual inspections in sites with high APD and three in sites with low APD.

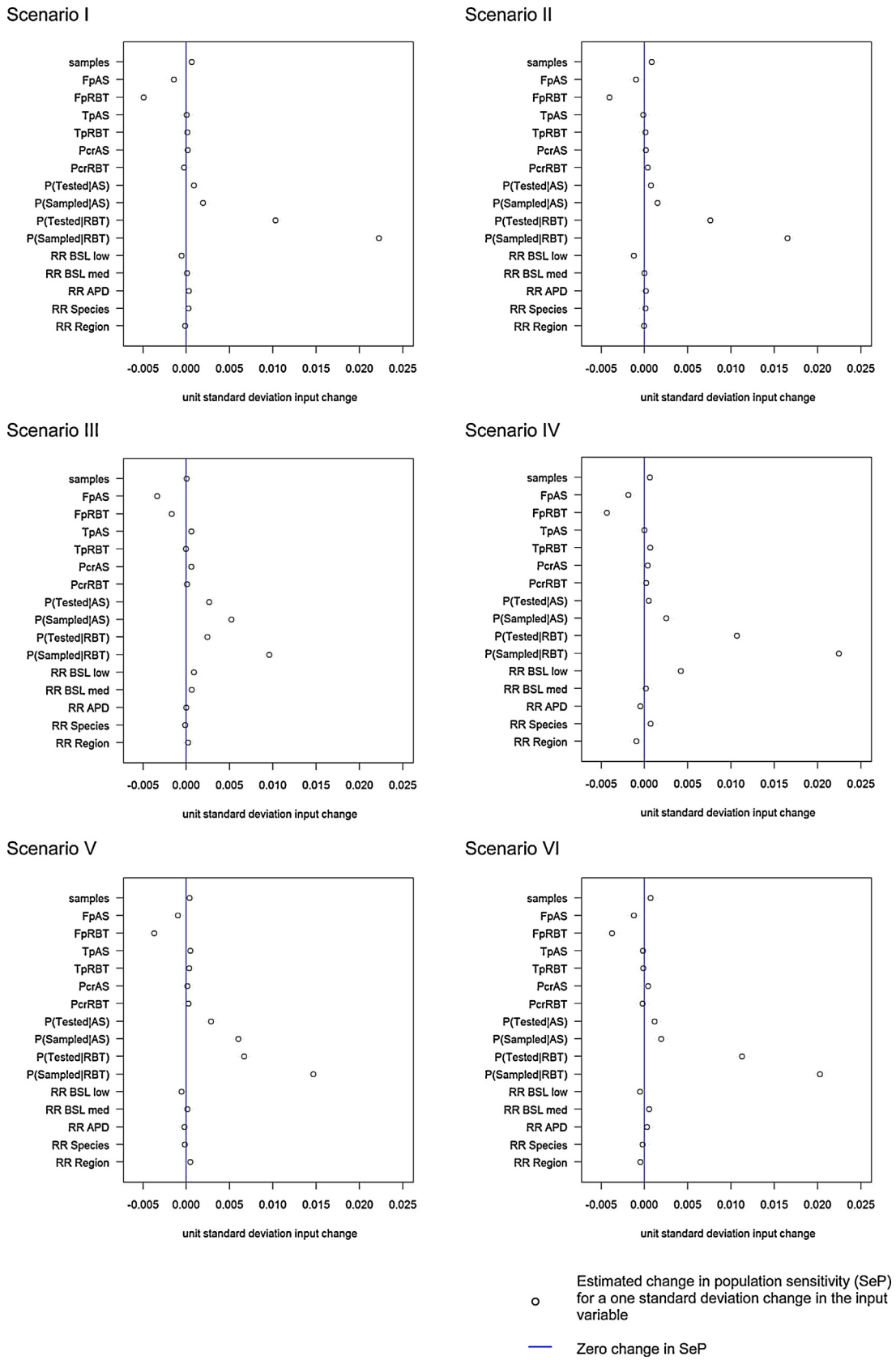


**Fig. 3.** Sensitivity analysis of model input parameters shown by estimated change in population sensitivity (SeP) for a one standard deviation change in the input variable for each of the six surveillance scenarios: Scenario I with six annual inspections in all marine RBT and AS sites, Scenario II with three annual inspections in in all marine RBT and AS sites, Scenario III with six annual inspections in RBT sites and one annual visit in AS sites, Scenario IV with six annual inspections in sites in Region South and three in sites in Region North, Scenario V with six, four and zero inspections in sites with low, medium and high BSL, respectively, and Scenario VI with six annual inspections in sites with high APD and three in sites with low APD. Model input variables included number of samples (samples), probability of clinical signs in VHS uninfected AS and RBT (FpAS and FpRBT), probability of clinical signs in VHS infected AS and RBT (TpAS and TpRBT), sensitivity of real-time PCR test in AS and RBT (PcrAS and PcrRBT), probability that AS and RBT samples are tested given that the population is infected and samples submitted (P(Tested|AS) and P(Tested|RBT)), probability that AS and

while maintaining the high detection level. BSL was included in the model, despite lack of data, in order to demonstrate the potential effect of low *versus* high biosecurity levels. The level of biosecurity will be influenced by the daily management, presence of wild fish, movement of fish between sites, *etc*. A future standardized system including biosecurity (*e.g.* a biosecurity index) would facilitate the identification and ranking of sites according to biosecurity and management practices.

SeP and PFree were estimated for six different scenarios in order to illustrate the usefullness of the model as a supporting tool for evaluating possible changes in the surveillance strategy. PFree above 95% was achieved after only three months for the two scenarios with the highest frequency of annual inspection. The time period (months) needed to achieve a PFree >95% increased when the frequency of inspection decreased, even though inspections were targeted towards high risk groups. Scenario I reflects the current surveillance scheme in Norway, where all marine grow-out salmonid sites are inspected every other month. Scenario II reflects that the consequence of reducing the inspection frequency by 50% increases the time until a PFree >95% from three to seven months. Scenario III showed the effect of reducing inspection frequency of AS sites to once per year, while the inspection frequency of RBT sites was six per year. Even though RBT is considered to have a higher VHS risk than AS (RR = 6.5, Table 2), Scenario III was the only scenario that did not achieve a PFree of 95% within 12 months. This result can be explained by the low number of RBT sites, which account for only 9% of active salmonid sites in 2014. Scenario IV explored the effect of prioritizing sites in Region South by having six annual inspections, rather than the three inspections in Region North. In this scenario, a PFree >95% was achieved after only three months. This scenario can be considered as the most cost efficient strategy with respect to VHS, because the frequency of inspection is reduced to three per year in Region North. The result can be explained by the fact that the majority of sites, and in particular RBT sites, are located in Region South, and the lower RR of region North *versus* South. In Scenario V, the impact of targeting sites having low or medium BSL was explored, and a PFree >95% was achieved after 10 months. However, this scenario requires more resources in planning, identification, and selection of sites with low or medium BSL, and a reliable system for characterizing sites according to their BSL. Scenario VI explored the effect of prioritizing sites with high APD by having six annual inspections in high APD sites and three in sites having low APD. With this strategy a Pfree >95% was achieved after 5 months, and included fewer monthly inspections than Scenario IV.

We used a conservative estimate for the sensitivity of the real-time PCR test (0.9, Jonstrup et al., 2013) in our model. If we had used the estimates from Warg et al. (2014, 0.96), the probability of freedom would marginally improve and the time to achieve 95% freedom would be slightly reduced (result not shown).

The use of production statistics allowed robust calculations of population proportions and mortality, while RRs of the other input parameters were informed estimates. The RRs for APD and BSL were based on the authors' opinion due to lack of data. They were included to allow for evaluation of these factors as potential factors for targeting surveillance in the future. Thus, the scenario modelling technique, together with stochastic simulation, allowed for the inclusion of uncertainty in the model, and proved useful for exploring and analysing the surveillance system.

Results from the sensitivity analysis of the most influential input variables on model outputs (SeP estimates) highlighted the importance of having competent personnel carrying out clinical inspection in farm sites. The analysis identified the probabilities that RBT are sampled and that the samples are tested, given that the site is infected, as the most important factors that drive the model, and indicated that these are factors important to work on to obtain more accurate and reliable estimates, and that measures should be put in place to improve these probabilities. Sensitivity analysis of the remaining factors showed comparatively minor or non-significant effects, suggesting that the present model is less sensitive to changes in these input parameters, at least within the range of values used.

The former Norwegian surveillance programme for documenting VHS freedom required that a large number of samples were tested annually, underlining that a more cost-effective surveillance strategy was needed. The present model, although theoretical, demonstrates the potential of a risk-based approach performed by professionals already in place doing on-site fish health services for the industry. For all scenarios evaluated, the laboratory costs of this system are less than a tenth of the laboratory cost of the original programme.

It is important to note that the current model only concerns VHSV strains that may cause clinical signs of a systemic infection. VHS has a large host range and there is considerable variation in the pathogenicity of VHSV to different fish species (Skall et al., 2005; Office International des Epizooties, 2012). Subclinical infections could pose a problem for detection, and this model does not provide any estimates of the probabilities that low pathogenic strains will be detected.

The model provides a platform for surveillance of other exotic viral infectious diseases in marine salmonid farming in Norway, if they share similar risk factors. An adaptation of the model to the freshwater phase (ponds and rivers) would require more modifications as the pathways of pathogen introduction differ for marine and freshwater sites (Oidtmann et al., 2011, 2013). A system of risk ranking of farm sites, taking into account the risk for introduction and spread of disease, is acknowledged by the current EU directive 2006/88/EC. Such risk ranking would provide useful input and improve our scenario tree model. Future improvements could also be made by conducting studies to obtain more precise estimates of RRs and the probability that fish are sampled, submitted and tested given that a population is infected.

## 5. Conclusion

Model results indicate that the current surveillance system based on routine inspections by the FHS has a high capability for detecting VHS in marine farmed salmonids. The system has been running in Norway for many years and provides a high probability of freedom from VHS (PFree >95%). The sensitivity analysis showed that the probabilities that RBT are sampled and tested were the two most influential input variables. The model provides a supporting tool for evaluation of potential changes in the surveillance strategy, and can be viewed as a platform for similar exotic viral infectious diseases in marine salmonid farming in Norway, as they share similar risk factors.

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RBT samples are sampled given that the population is infected (P(Sampled|AS) and P(Sampled|RBT)), relative risk (RR) of low and medium compared to high biosecurity level (RR BSL low and RR BSL med), RR of high compared to low area production density (RR APD), RR of RBT compared to AS (RR Species), and RR of region south compared to region north (RR Region).

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